

29 de Mayo de 2015

Estimados Colegas,

Paso a detallar las últimas novedades respecto de los caballos argentinos sacrificados en Chile por una supuesta serología positiva a Muermo.

- 1) Datos aportados por la persona del laboratorio que realizó los tests en Chile, la Dra A. Pezoa:

De: Teótimo Becú - Clínica Equina SRL [<mailto:t-becu@redsarmiento.com.ar>]

Enviado el: martes, 26 de mayo de 2015 19:22

Para: Alexza Pezoa Adasme

Asunto: conectados

Estimada Dra Pezoa,

Quedamos conectados !

Estuve leyendo el trabajo alemán de Khan y Neubauer, y allí explica que la ESPECIFICIDAD del antígeno del USDA es del 100 %, mientras que la de los antígenos alemanes es del 97 %, lo que quiere decir que de cada 100 caballos, obtienen 3 FALSOS POSITIVOS, que es mucho ¡!.

Habría que ver si estos 3 caballos no eran simplemente falsos reactores. Podría decirme por favor cuantos caballos por mes, o por año, controla con el test de Muermo, y que tasa de positividad obtiene ?

Le envío un cordial saludo,



Teótimo Becú, M.V.

CLINICA EQUINA SRL

Director

Laboratorio de Diagnóstico

Laboratorio de Producción de Biológicos

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De: Alexza Pezoa Adasme [<mailto:alexza.pezoa@sag.gob.cl>]

Enviado el: Miércoles, 27 de Mayo de 2015 09:58 a.m.

Para: Teótimo Becú - Clínica Equina SRL

Asunto: RE: conectados

Estimado Dr. Becú.

Hay diferencia de sensibilidad y especificidad entre antígenos. La sensibilidad del antígeno NVSL es de 62.19%, lo que me parece más preocupante que diferencia de especificidad.

Efectivamente la FC puede tener falsos positivos, pero por esto mismo se enviaron las muestras a Alemania a confirmación por Western blot. Las reacciones cruzadas del WB se acotan a *B. pseudomallei*.

En estos momentos no tengo un número de análisis al año, lo puedo buscar, pero por lo pronto le informo que esta es primera vez que tenemos positivos.

Saludos cordiales,

Alexza Pezoa Adasme
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Unidad de Bacteriología
Servicio Agrícola y Ganadero
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De: Teótimo Becú - Clínica Equina SRL [<mailto:t-becu@redsarmiento.com.ar>]

Enviado el: miércoles, 27 de mayo de 2015 11:44

Para: Alexza Pezoa Adasme

Asunto: RE: conectados

Dra, entiendo que el antígeno que Ud utiliza, el CC.pro alemán, es un whole cell antigen, o sea diferente al que describe la OIE, que es un filtrado compuesto principalmente por LPS.

Es esto así ?

De: Alexza Pezoa Adasme [<mailto:alexza.pezoa@sag.gob.cl>]

Enviado el: Miércoles, 27 de Mayo de 2015

El antígeno Cc.pro está validado, probado y recomendado por el laboratorio de Referencia Alemán (FLI). Creo que si no siguiera las recomendaciones de la OIE no entregaría su certificado ni tampoco sería usado por FLI o por ANSES (laboratorio referencia para la UE).

Saludos,



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NOTA: aqui queda confirmado que el antígeno utilizado por el laboratorio Chileno es el C.C.-PRO, alemán, de un laboratorio privado. No queda claro si el mismo es fabricado bajo las recomendaciones de la OIE.

2) Comunicación con el Dr H. Neubauer, autoridad del FLI (Friedrich Loeffler Institute, Germany), y referencia de la OIE para Muermo:

De: Teófilo Becú - Clínica Equina SRL [<mailto:t-becu@redsarmiento.com.ar>]

Enviado el: Domingo, 24 de Mayo de 2015 09:00 p.m.

Para: 'heinrich.neubauer@fli.bund.de'

Asunto: Glanders serologic tests

Dear Professor Neubauer,

I'm writing to you because we need some help about an issue in regard to Glanders serology. I hope that this is OK for you and you don't mind.

Our Lab is officially authorized by Argentinian Agriculture to run almost all the sera samples of the horses to be exported or imported. This tests includes the following.

- EIA (AGID)
- Piroplasmosis (cELISA, CFT and IFAT)
- Dourine and Glanders (CFT)

We keep doing this work for the last 25 years, and so far, after test several thousands of horses per year, we almost did not have had problems with rejected reacting argentinian horses in other countries; just very few in more than 80.000 exported horses.

As you well know, the CF test for Glanders has been successfully been used by the USDA in order to prevent Glanders infected horses to get into USA. The same happened with Argentina and other countries that regularly purchase the same CF Glanders reagents from the NVSL.

However, we know that sometimes the CF might give false results, with the consequent economic lost for the horse owners. Nevertheless, in the overall, the Glanders CF test has proven to be a good test !

Some years ago, Dr J. Katz, from the DBL-NVSL-USDA, required to our lab to collaborate with him in the validation of a coordinate Piro-Dourine-Glanders CELISA test. The test was nice, but for some reason the NVSL never adopted it as their official test. The exception was for the Piroplasmosis, but they start to use the commercial VMRD cELISA kit, which was easier to use, and more reliable.

The problem:

Chile required official negative Coggins and cELISA piro for the horses that have to be imported, but for some unknown reason they tested a group of 27 Argentinian Thoroughbred horses, and apparently they found 3 POSITIVE to Glanders test (I ignore what Glanders serologic test they are currently using). We immediately test the 3 sera of these horses, and we found them as NEGATIVE. On the other hand, SENASA (Government lab) checked the same samples, and they also found them NEGATIVE.

The Chilean Government sent the 3 samples to Germany, and the lab in Germany obtained also POSITIVES results. I ignore yet what test did they use, but the Chilean authorities said that “ in Germany they use the only reliable Glanders test, with more sensivity than the USA test”.

Based in this findings, they KILLED the 27 horses !. No clinical signs at all. Argentina is free of Glanders, we never have had clinical cases, noR true POSITIVE serology.

Today I´ve read your paper about your new iELISA for Glanders, with 2 recombinant proteins, which apparently avoids the false positive cases (specially those due to B. pseudomallei), and have a higher sensivity than the CFT.

Questions:

- Were these samples from Chile tested in your lab ?, with the CFT or with iELISA ?
- In your paper, did you test just 123 horses with this iELISA ? Is it fully validated ? Is it OIE approved as a prescribe test for international movement of horses ?
- What do you know about the differences in specificity and sensivity between the CFT and your iELISA test ?
- Which test is officially using Germany for the imported horses ? CFT? Or iELISA ?
- Are also the German labs using their CFT with a different antigen than the crude bacterial LPS antigen that the USDA currently use ?
- Are you aware what are doing other countries in regard to the Glanders test ?, I mean, are they all using the classical LPS crude antigen, or they are moving to other tests ?

We are waiting for official info from Chile about what Glanders test are they currently use. Anyway, we are very concern with the discrepancy of our results, we had check for decades many thousands of horses that have travelled to USA and Europe without problems !! This may become a serious problem, if different countries use different tests with different antigens. And more importantly, we don´t want a false record of 3 Glanders POSITIVE horses !.

Thanks a lot in advance for the help, and sorry for the long letter. I´ll look forward for your response.

Sincerely yours,



Teótimo Becú, M.V.
CLINICA EQUINA SRL
Director
Laboratorio de Diagnóstico
Laboratorio de Producción de Biológicos
Tel-fax: 02478-481658/481764

De: Neubauer, Heinrich [mailto:Heinrich.Neubauer@fli.bund.de]

Enviado el: Jueves, 28 de Mayo de 2015 08:27 a.m.

Para: 'Teótimo Becú - Clínica Equina SRL'

CC: Elschner, Mandy

Asunto: AW: Glanders serologic tests

Hello,

I will answer your questions as good as I can.

None of the newer tests is validated or OIE certified. The number of positive horse samples (culture or PCR positive) is so high that nobody was able to collect this number till now - we just try to involve various researchers from different countries to add their positives to our serum collection and to let us test their diagnostics (including the Indian one) in Jena. This project will hopefully be financed by the OIE and hopefully the tests will be OIE accepted then. CFT remains the test for the OIE to be used for international trade. We use here the Western Blot as described by Katz et al - you know him obviously. This test has been used in various countries and during various outbreaks and it is a very sensitive and specific test in European and Middle Asian settings - you can find the publications in pubmed if you like. We adopted our German regulations accordingly and combine CFT and WB for diagnosis. We test all sera which we find CFT positive also by WB when we act as OIE reference lab. I cannot tell you whether those sera were investigated by us or not - you have to ask your Southern American partners to get this information. There are several CFT antigens on the market and we have not tested all these antigens and of course we do not know all the producers. OIE validates techniques like the CFT - it does not give you a producer name or recommends a special company: this is simply not within its mandate. We published our way to do the CFT and for standardization reasons we try to buy as many components from QM certified companies to minimize errors. This is fair in my eyes. Again for international trade CFT is the test to do and it simply relies on 'field' strains and crude preparations of them. The old USDA antigen had some problems, we supplied them with our seed strains and we hope the CFT antigen is more compareable to the one which is used in Europe. I hope this info helps a little and we are really prepared to discuss the problem. Please contact us again. We are working on international standards but it takes time and there is no real funding available for such work.

Prof. Dr. Heinrich Neubauer
Institutsleiter
Institut für bakterielle Infektionen und Zoonosen

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Von: Teótimo Becú - Clínica Equina SRL [mailto:t-becu@redsarmiento.com.ar]

Gesendet: Montag, 25. Mai 2015 17:04

An: Neubauer, Heinrich

Betreff: RE: Glanders serologic tests

Dear Dr Neubauer,

By mistake I've told you that the paper about the Glanders IELISA with 2 recombinant proteins was yours, when actually the author is Vijai Pal, India. Sorry about that.

I read the abstracts of your papers about Glanders serology, but couldn't get the full text yet.

Thanks,

T. Becu

Aqui en este mail, en azul están mis preguntas, en rojo las respuestas del Dr Neubauer, y en verde una traduccion resumida:

Von: Teótimo Becú - Clinica Equina SRL [<mailto:t-becu@redsarmiento.com.ar>]

Gesendet: Donnerstag, 28. Mai 2015 17:38

An: Neubauer, Heinrich

Betreff: RE: Glanders serologic tests

Dear Dr Neubauer,

Thanks a lot for your reply and your time. It's an honour to have the opportunity to discuss this topics with a world reference as you.

Just to be sure that I have understand, and some additional questions:

- When you say that none of the newer tests is validated or OIE approved, you are talking just about the ELISAs, right ? Specifically those cELISA and iELISA described in your paper "Prevalence-dependent use of serological tests for diagnosing glanders in horses", right ?
No ALL (Aqui se entiende que ninguno de los test nuevos de Muermo esta validado por la OIE)
-
- Regarding the WB, yes, it is a very sensitive test, but, if you use the same antigen in the WB that you are using in the CFT, the specificity is not going to be higher, compared with the CFT, because the antigen is the same. Am I wrong ?

It is a different preparation, because the WB is detecting only LPS banding patterns directly by eye - the CFT does detect antibody reaction to antigens via the hemolytic system indirectly: you cannot directly compare both techniques and whatever is reactive in it. Therefore, you have sensitivity and specificity giving you a range of what is to be expected to be false positive or negative and these samples can or cannot be the same ones in the different test. So a test based on the same seed strains i.e. antigens can have a higher sensitivity or specificity than another one using the same seed strains. You have to validate every test therefore applying the same standards.

(El Western Blot sería aparentemente más sensible y específico que el CF test, como se demostró en un trabajo de Khan, Neubauer y otros en 2011....se usa como confirmatorio, pero no es practico para correr muchas muestras)

-
- Where are processed the samples for Glanders of the imported horses to Germany ?, in the Loeffler Inst ?

No we are a higher governmental body and it is not our job to do this - indeed most private labs which have been certified do over these tests on hire. We do help the Länder if they have special problems not to be solved by the regional Länderlabs.

(Los tests oficiales de Muermo en Alemania NO son procesadas por el FLI (Friedrich Loeffler Institute, Lab de referencia de la OIE para Muermo).Al igual que en la Argentina, se procesan en laboratorios privados certificados)

-
- The OIE Manual describes in detail the CF test technique and the antigen production Glanders procedures that should be used. As you well know, this is a bacterial culture filtrate, heat inactivated crude antigen, which mostly consist in LPS. Is this right ?.

We have not checked for that so I cannot give you this information based on my experience.
(Esto es un poco desconcertante, pues lo que quiero definir es que QUEDE CLARO que el Manual de la OIE describe en forma específica como debe ser procesado el antígeno de Burkholderia mallei, pues esto es crítico para saber si el antígeno alemán CCPro que usó Chile, CUMPLE O NO CON LO QUE DICE EL MANUAL DE LA OIE. Como toda respuesta, no puede darme esa información)

Therefore, the OIE cannot of course recommend you a certain company, but all the official labs in the world involved in Glanders serology have to follow these OIE indications, both for the CF technique and antigen production, in order to guarantee the standardization of the procedures, and to assure that there will also be similar results in different international labs. Am I wrong ?

This is not exactly correct - these are standards to be followed to guarantee a certain quality of diagnosis for international trade but the countries are free to negotiate own standards or they simply have higher standards and do not accept the OIE standards and so on. It is not a perfect system but the best one available for the moment of course.

(Aqui de nuevo sorprende un poco la respuesta. Ante la pregunta de que si los laboratorios oficiales de todo el mundo deberían seguir las indicaciones del Manual de la OIE, a fin de garantizar una estandarización de los métodos, y asegurar que los diferente laboratorios obtengan resultados similares, el Dr Neubauer, responde que los países son libres de negociar sus propios estándares, o simplemente establecer estándares mas exigentes y no aceptar los de la OIE. Yo pregunto: para que sirve entonces la OIE ¿??, si cada uno va a establecer los estándares que se le da la gana ?)

- Therefore, is/are the official german lab that runs the Glanders serology for the imported horses, using the exact procedures describe in the OIE Manual ?, or simply the antigen production of the CF german antigen is different, and this is OK ?

I cannot answer this question - German labs are overseen by the Länder and the Accreditation / Certification body of Germany. These institutions look very exactly at the SOPs and systems used in the lab. FLI is the body to prove every batch of diagnostics to be used for glanders diagnostics what ever the test principle may be. So we guarantee for the quality of the products used what ever the test principle may be.

(Aquí, ante la pregunta de si los laboratorios oficiales de Alemania que procesan las muestras de Muermo utilizan rigurosamente los procedimientos indicados por la OIE, el Dr Neubauer dice que no puede responder esta pregunta. Dice que el FLI controla todas las partidas de antígeno de Muermo que son producidas, para garantizar la calidad del producto. Pero no contesta si se siguen los lineamientos de la OIE)

- Is the C.C. pro Glanders antigen validated and approved by the OIE ?. This is crucial.

This you have to ask the OIE directly because they do not tell us. It should be info available from their homepage. At least I was not involved in such a process - which of course does not mean it is impossible.

(ESTA PREGUNA ES CRUCIAL !!, pregunto si el antígeno CCPro se encuentra validado y aprobado por la OIE. Respuesta, hay que preguntarle a la OIE.(aclaro que su laboratorio es referencia de la OIE para Muermo). El Dr Neubauer dice que el no estuvo involucrado en dicho proceso.

- I've read in one paper (from you) that one reason to explain the low sensivity of the USDA Glanders antigen vs the C.C.Pro antigen, is the old Chinese strain use in the USA atg, that may have lost its antigenicity or lacking some epitopes. On the other hand I've read that the C.C. Pro is a whole cell wall antigen. Is this right ?

Yes - I speculated that epitope loss could be the reason for low sensitivity. We have not analysed the ccpro antigen as such I cannot tell you what it is. We can only tell you that we test the batches we get from them and allow them to purchase them in Germany.

(Aquí me confirma que una de las razones por las cuales se especula sobre las causas de la diferencia en sensibilidad entre el antígeno del USDA y el CC-Pro alemán, es la pérdida de ciertos epitopos. No sabe cual es la naturaleza del antígeno CC-Pro)

- Message to home: is it acceptable to use different Glanders antigens in the international movement of horses ?

I think this is done already and I cannot see a regulation which would or could avoid this.

(Ante mi pregunta de si es aceptable que se utilizen diferentes antígenos en diferentes países en el movimiento internacional de caballos, contesta que es lo que ya ocurre, y no imagina una regulación que pueda evitar esto. Aquí también esto desconcierta: para que sirven los parámetros establecidos en el Manual de la OIE ?)

And finally, yesterday I call my friends in the DBL, NVSL, and they told me that they are using the C.C. Pro antigen !!!, so I'm exposed to obtain discrepant results because I'm using their antigen !, this is becoming crazy....

It is better to use the same antigen worldwide - the next step would be ring trails to make sure that the technique works in the different labs.

(Aquí dice que sería mejor utilizar un mismo antígeno en todo el mundo....., y realizar pruebas que aseguren que las técnicas funcionan en los diferentes laboratorios)

In regard to the international project to validate the cELISA/iELISA, please don't hesitate to require our help, if there's a way that we may help you. We run 1.800 glanders samples per year, but we receive many more thousands for other serology tests. Since Argentina is a free Glanders country, we don't have positive samples, but we might get a lot from Brazil. If you need us to run your ELISAs tests here in our lab, please, just let me know. We would be very happy to cooperate in the project.

Thank you - I will come back to you and if it is possible I really would ask for several hundreds of sera of different origin. What are the conditions to get them?

Ciao Heiner

Sorry for my long letter; your opinions are crucial for us.

Best wishes,



Teótimo Becú, M.V.
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3) Que dice la empresa alemana C.C.-Pro sobre su antígeno ?

De: Teótimo Becú - Clinica Equina SRL [mailto:t-becu@redsarmiento.com.ar]
Enviado el: Jueves, 28 de Mayo de 2015 04:15 p.m.
Para: 'info@c-c-pro.com'
Asunto: glanders antigen

Am 28.05.2015 21:15, schrieb Teótimo Becú - Clinica Equina SRL:
Dear Sirs,

We are interested in the cc Pro CF Malleus-KBR Antigen 1:160. Please, I would like to know:

If you have got the product in stock.

The price.

If this antigen is suitable for the Glanders CF test.

If this antigen is validated and approved by the OIE to be used in the serology for international movement of horses.

If this antigen is produced following the OIE Manual description of how to produce the Glanders antigen.

If this is a LPS antigen or a whole cell antigen.

Thanks in advance.

Regards,



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De: ccpro GmbH [mailto:info@c-c-pro.com]

Enviado el: Viernes, 29 de Mayo de 2015 11:36 a.m.

Para: "t-becu@redsarmiento.com.ar >> Teótimo Becú - Clinica Equina SRL"

Asunto: RV: glanders antigen

Dear Dr. Becu,

- at the moment we are a little bit short, but we can supply smaller quantities
- The product is intended for the use in CFT .
- It is approved by the german goverment institution FLI, see accompanied certificate.
- The glanders antigen has the order nr. VD-0013-N, **10 ml/ €542,-.**

It is not supplied in working strenght, you should dilute it 1:39.

As you may read in the -User´s instructions- it is an **extract**, not whole cells.

We don´t comment the production procedure, so we can´t answer if it is conform with OIE.

We hope that we could answer your questions.

Kind regards,

--

Bruno Stübchen

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NOTA: aquí de nuevo no hay confirmación sobre si el antígeno se produce bajo los lineamientos de la OIE, o no

4) Algunas reflexiones finales:

- Argentina es un país libre de Muermo, lo atestigua la ausencia total de casos clínicos, más la evidencia de la exportación de más de 80.000 caballos a todo el mundo en los últimos 25 años, sin haberse registrado jamás ningún incidente como el sucedido en Chile.

- El SAG chileno dá como positivos los caballos argentinos basado en un test que utiliza un antígeno alemán, el C.C.-PRO del cual no hay evidencias que sea producido bajo lo que prescribe el Manual de la OIE.

- Tengo entendido que el SAG envió las muestras al FLI alemán, confirmando los positivos, pero no disponemos de la documentación que pruebe esto.

- En Argentina todos los sueros de los caballos en cuestión arrojaron un resultado NEGATIVO, utilizando un antígeno, del USDA, preparados bajo los lineamientos indicados por la OIE.
- De acuerdo a una investigación realizada comparando diversos antígenos de Muermo, este antígeno alemán dio una especificidad del 96,5 %, esto quiere decir que 3 o 4 caballos de cada 100 pueden dar FALSOS POSITIVOS. El del USDA dio un 100 % de especificidad.
- Las consultas realizadas tanto al DR Neubauer, del laboratorio Friedrich Loeffler Institut, como a la empresa C.C.-Pro, no indican que este antígeno sea preparado de acuerdo a las indicaciones del Manual de la OIE.
- Esta diferencia de antígenos puede por supuesto dar lugar a diferentes resultados, y esto ser el origen del problema !
- El sacrificio inmediato de los 27 caballos argentinos, sin contrapruebas suficientes, sin evidencia alguna de sintomatología clínica, sin aislamiento del germen causal, sin necropsias, aparece, cuando menos, como una medida apresurada.
- Este Lunes hemos sido invitados por las autoridades de SENASA a una reunión para tratar el tema. Urge que SENASA se expida oficialmente, y que se envíen oficialmente los sueros al USDA a fin de verificar los resultados.
- Estamos en conversaciones con el USDA, con el Gluck Center de Kentucky y con las autoridades de SENASA para definir con que antígeno deberían hacerse las pruebas de Muermo de aquí en más !



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